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Nitrogen availability influences microbial reduction of ferrihydrite- organic carbon with substantial implications for exports of iron and carbon from peatlands

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Abstract:

While iron (Fe) has been proposed to constrain dissolved organic carbon (DOC) export by forming precipitation (Fe-OC) in peatlands, uncertainties remain about the potential interactions between Fe and nitrogen. Such interactions are important for Fe and carbon exports as they can dissolve the Fe-OC through Fe reduction. Here we studied the reduction of ferrihydrite-OC (Fh-OC) following incubation with microbes from agricultural and natural peatlands under differing nitrogen availability, with high-throughput sequencing to identify microbial mechanisms. Our results showed that in agricultural peatlands, high nitrogen levels (>100mg kg^{-1}) modified the composition and abundance of iron-reducing bacteria, increasing reduction rates of Fh-OC (0.09-0.20 kg day^{-1} ha^{-1} of Fh-OC) above the low N treatments (<100 mg kg^{-1}) ($p<0.05$). However, reduction rates of Fh-OC and iron-reducing community in natural peatlands were far less affected. Our findings suggest that N-induced microbial reduction of Fh-OC may create the potential for greater iron and carbon exports from agricultural peatlands to aquatic systems.

Key words: Iron-reducing bacteria; ferrihydrite-organic carbon; nitrogen; peatlands; agriculture.

1. Introduction

Peatlands represent a global major carbon (C) store, and a dominant source of dissolved organic carbon (DOC) to aquatic systems (Fenner et al., 2007). Climate change and human activity have been found to increase DOC export to freshwater from peatlands (Freeman et al., 2001; Evans et al., 2005; Fenner et al., 2007; Bjorneras et al., 2017). Iron (Fe) has been noted to constrain DOC export through coprecipitation or adsorption, forming immobilized complexes (Fe-OC) in peatland (Riedel et al., 2013), with studies showing that Fe stabilizes 8-17 % of organic carbon (Wen et al., 2019; Zhao et al., 2019). However, recent studies also report that increased DOC concentrations are synchronous with dissolved Fe concentrations in peat catchment (Ekstrom, et al., 2016; Bjorneras et al., 2017).

Under global warming, rising temperatures could stimulate Fe reduction (Knorr, 2013), leading to DOC and Fe release from Fe-OC (Pan et al., 2016). Peatlands contain an abundance of phenolics, which contribute to forming stable dissolved complexes with Fe(Ⅲ) or Fe(Ⅱ) (Wan et al., 2018). These complexes could offer protection against forming Fe-oxyhydroxide precipitation, further supporting the unimpeded transport of Fe and carbon from peatlands to aquatic ecosystems (Krachler et al., 2015; Wan et al., 2018). There is further possible influence from anaerobic ammonium oxidation coupled to iron reduction (Feammox), which is known to be widespread in natural anaerobic conditions (Yang et al., 2012; Ding et al., 2017). Moreover, long-term N fertilization in soil has also been found to enhance Fe reduction rate (Ding et al., 2015). Peatlands belong to nutrient-poor ecosystems (Bragazza et al., 2006), which are threatened by increased atmospheric nitrogen deposition (Bragazza et al., 2006; Li et al., 2019). Furthermore, about 14-20 % of peatlands have been drained for agriculture worldwide (IPS, 2008), in which fertilization significantly increases N content (Ruckauf et al., 2004). It is clearly important to determine the extent to which increased N availability will affect reduction of Fe-OC, due to the consequent implications for iron and DOC export from peatlands to aquatic system.

The incubation experiment with microbes from environmental soil and synthesized ferrihydrite or ferrihydrite-OC (Fh-OC), has been widely applied in studies of iron biogeochemistry as a way of minimising wider environmental influences on the targeted iron transformation processes (Zhuang et al., 2015; Cooper et al., 2017). Here, we observed the Fh-OC reduction with microbes from agricultural and natural peatlands under different N availability and used high-throughput sequencing to reveal microbial mechanisms. According to N availability in agricultural and natural peatlands, we set up two high N (>100mg N kg^{-1}) and four low N (<100mg N kg^{-1}) treatments. We hypothesized that (1) high N contents would increase microbial reduction of Fh-OC in agricultural peatlands only as long-term N application may increase microbial N demand. (2) Increased N availability would increase microbial reduction of Fh-OC in natural peatlands.

2. Materials and method

2.1. Experiment design

Soil samples were collected from Jinchan Peatland, a temperate fen in Northeast China (42°21′-42°22′ N, 126°21′-126°22′E), where areas of peatland have been converted to paddy fields since the 1960s. In general, N fertilizer (urea) is applied mid-May, and throughout June for rice growth, totalling 260 kg N ha^{-1} $year^{-1}$. In contrast, P fertilizer is applied in mid-May only, amounting to 70 kg P ha^{-1} $year^{-1}$ (Shi., 2019). Soil properties in agricultural and natural peatlands are shown in table 1. Atmospheric N deposition in northeast China is about 1.4 g N m^{-2} yr^{-1}, which is significantly higher than in other global areas at a similar latitude (< 0.6 g N m^{-2} yr^{-1}) (Li et al., 2019).

According to Zhang et al. (2016)'s field investigation data, in August 2018, we selected eight random sample sites in flooded agricultural and natural peatlands. After removing litter and vegetation, we collected three random soil cores using a soil core sampler at 0-15 cm depth each site, then mixed and transported to a laboratory on ice under anoxic conditions (N_2). We extracted microbes from each site within 72 h using the standard protocol of Cooper et al. (2017). Fresh soil (equal to dry mass 2.0 g) was added to sterile, anoxic 100 ml of 0.85 % NaCl solution and 8 g sterile glass beads in serum bottles (eight replicates), and the slurry was shaken at 4 °C overnight. After centrifugation at 900 g for 5 min, the supernatant was transferred to fresh sterile tubes and centrifuged again at 1200 g for 10min. Finally, the solid phase was resuspended in 10 ml anoxic 0.85 % NaCl solution and we used microscopy (XDS-2BI, China) to confirm the presence of microorganisms in the suspension.

Fh-OC was synthesized as described by Pan et al. (2016) with minor modifications. A water extract of freeze-dried soil litter, from natural peatlands with 30:1 ratio, filtered with 0.22 μm and diluted in 200 mg L^{-1} DOC, and as a molar ratio 1:1.04 of C with Fe ($FeCl_3$) observed in the field (4 g kg^{-1} DOC and 18 g kg^{-1} SRO). While constantly stirring, 0.1 M NaOH was added to pH6 then centrifuged 5000 rpm and the supernatant decanted (repeated three times). Solid Fh-OC with final C/Fe 0.35 was formed after freeze-drying. Based on N

availabilities in agricultural and natural peatlands, we set up high N (as 100, 400 mg N kg^{-1}) and low N content (as 0, 1, 10, 50 mg N kg^{-1}) treatments. The 40 ml medium consisted of 30 ml L^{-1} of sterile 1 M $NaHCO_3$ (autoclaved, CO_2), 10 ml L^{-1} Wolfe's vitamin solution (ATCC, 1957) and 10 ml L^{-1} modified Wolfe's minerals (ATCC, 1957), a combination of filter-sterilized electron donors (sodium acetate, 2 mM; sodium lactate, 2 mM; and glucose, 2 mM), 1 mM KH_2PO_4, 80 mg Fh-OC, 25 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic (Hepes, pH=6), 2.5 ml bacterial suspensions (equal to 0.5 g dry soil) and NH_4Cl as nitrogen treatment in 50 ml vials (Cooper et al., 2017). The vials were sealed with a rubber septa and aluminium crimp caps after being purged with ultrapure helium for 20 min at a rate of 10 ml min^{-1}. All treatments were performed in triplicate, at a constant temperature of 25 °C, under dark conditions. Reduction of Fh-OC was monitored as Fe (II) every 24 h using the phenanthroline assay (Cooper et al., 2017). At the end of incubation (240 h), we sampled the microbial community.

2.2. DNA Extraction and 16S rRNA Gene Sequencing

DNA from the enrichment culture was collected by centrifugation (8000 rpm and 4 °C for 10 min) and then extracted using a Power Soil ™ DNA isolation kit (MO BIO Laboratories, USA), according to the manufacturer's instructions. Bacterial 16S rRNA gene fragments were amplified from DNA samples using the primers F341 and R806 (Mori et al., 2014). Then the samples were sent to Novogene (Beijing, China) for amplicon sequencing using an Ion S5 XL platform. The bioinformatics analysis was performed following previously described methods (Yang et al., 2019). All raw sequences have been deposited into a NCBI Sequence Read Archive with the accession number PRJNA576710.

2.3. Statistical analysis

Linear regression was performed to identify reduction rate of Fh-OC over 240h, and we estimated microbial reduction rate of Fh-OC at field scale (kg day^{-1} ha^{-1}) based on soil bulk density and reduction rate of Fh-OC in cultures. Differences between treatments were evaluated by one-way analysis of variance. Significant differences between means were

established by Duncan test at $p<0.05$. These were performed by the statistical package SPSS23.0. Furthermore, we used the Functional Annotation of Prokaryotic Taxa (FAPROTAX) database to annotate microbial functional groups based on 16S rRNA sequences (Louca et al., 2016). Nonmetric multidimensional scaling (NMDS) based on Bray-Curtis distance was performed to predict iron-reducing bacterial composition by Canoco 5.0.

3. Results

With microbes from agricultural peatlands, low N treatments (0, 1, 10, 50 mg kg^{-1}) did not affect the reduction rates of Fh-OC during the 240h incubations, and these were significantly lower than those of the high N treatments (100, 400 mg kg^{-1}) ($p<0.05$), with the higher rate in 400 mg kg^{-1} treatment than in100mg kg^{-1} treatment ($p<0.05$). Interestingly, the 100 mg N kg^{-1} treatment triggered a more rapid rise of Fe (Ⅱ) from 120 h to168 h, and then the rate subsided (Figure 1a). However, with microbes from natural peatlands, we found little variation in the reduction rates of Fh-OC across the any of the N treatments (Figure 1b).

We also estimated the microbial reduction rates of Fh-OC at a field scale across the N treatments. Results showed that high N treatments significantly increased rates by 0.09-0.20 kg day^{-1} ha^{-1} compared with low N contents treatments in agricultural peatlands ($p<0.05$) (Figure 2), which were all significantly higher than all the N treatments in natural peatlands. Overall, average microbial reduction rate of Fh-OC (1.21 kg day^{-1} ha^{-1}) in agricultural peatlands was about 13 times more than that in natural peatlands (Figure 2).

By using the FAPROTAX database, the effects of N on iron-reducing bacteria composition were revealed by NMDS (Figure 3). Results showed that the 400 and 100 mg N kg^{-1} treatments induced a similar iron-reducing bacteria composition in cultures with microbes from agricultural peatlands. However, the composition in corresponding low N treatments were similar to those in all the N treatments in the natural peatlands. Furthermore, a heat map showed a shift in relative abundance of the key microbial functional groups (Figure 4). There was a noticeably high relative abundance of iron-reducing bacteria under 100 and 400 mg N kg^{-1} treatment in agricultural peatlands, and groups associated with the

degradation of aromatic-hydrocarbon, hydrocarbon, plastic, aromatic compounds, and fumarate reduction under 400 mg N kg^{-1} treatment in natural peatlands.

4. Discussion

Our findings support the hypothesis that high N can increase reduction rates, but only in agricultural peatlands. Iron-reducing bacteria composition from cultures under low N contents were similar to those in all the N treatments in natural peatlands. However, in agricultural peatlands, high N content resulted in a modified iron-reducing bacteria composition and increased relative abundance (Figure 3&4). These findings support previous observations that long-term N fertilization can change iron-reducing bacteria community in mineral soils and likewise increase iron reduction rates in paddy soils (Ding et al., 2015). Furthermore, reduction rate in 100 mg N kg^{-1} treatment was significantly lower than 400 mg N kg^{-1} ($p<0.05$), and reduction rate of Fh-OC tended to decrease from 168 to 240h (Figure 1a). This also implies that a shift of iron-reducing bacteria has a high demand in nitrogen, which then mediates reduction of Fh-OC. However, results disagree with hypothesis in natural peatlands, as iron-reducing composition and relative abundance were far less affected by N treatments in natural peatlands (Figure 3&4). Moreover, N-replete conditions (400 mg kg^{-1} N) could promote microbial groups associated with the degradation of aromatic-hydrocarbon, hydrocarbon, plastic and aromatic compound (Figure 4). These findings support previous studies that high N availability could increase phenol oxidase activity, a specialized enzyme for degrading recalcitrant materials in peatlands (Bragazza et al., 2006; Song et al., 2019). These also suggest that N is not the determining nutrient for iron-reducing bacteria during short-term incubations.

Although laboratory control experiments revealed the effects of single N on microbial reduction of Fh-OC, field soil conditions also influence the microbial composition and function (DeAngelis et al., 2010), which could determine the microbial reduction of Fh-OC. In our study, compared with natural peatlands, soil short-ranged iron, soil total phosphorus and soil bulk density increased, but soil organic carbon decreased in agricultural peatlands

(Table1). First of all, iron-reducing bacteria mainly use short-ranged Fe oxides (Lovley, 1987), which could influence iron-reducing bacteria composition and increase their abundance (Liu et al., 2019). This implies that a shift of iron-reducing bacteria composition is also related to field short-ranged iron contents. Secondly, P limitation is common in fens (Hill et al., 2014), while Li et al. (2019) also reported that P plays a key role in soil biochemical cycling in fens located in Northeast China. Therefore, sufficient P in incubated cultures might cause overestimation of microbial reduction rate of Fh-OC in natural peatlands as reduction of P limitation (Bongoua-Devisme et al., 2013). Thirdly, agricultural practice could increase peat bulk density and decrease soil organic carbon in drained peatlands (Kasimir-Klemedtsson et al., 1997). The reduction rates of Fh-OC appeared no different between cultures with microbes from agricultural and natural peatlands (Figure 1a&b), but average microbial reduction rate of Fh-OC (1.21 kg day^{-1} ha^{-1}) in agricultural peatlands was about 13 times more than that in natural peatlands at field scale (Figure 2). These also emphasize that the increased soil bulk density in agricultural peatlands can enhance total production of Fh-OC reduction per area. Although carbon contents and different organic carbon as electron donor could also influence microbial Fe reduction (Yang and Liptzen, 2015; Su et al., 2020), soil organic carbon (27.2%) is still at high levels in agricultural peatlands. Therefore, further work is needed on organic carbon composition and its effect on reduction of Fh-OC in agricultural peatlands.

Globally, 14-20% of peatlands have been drained for agriculture (IPS, 2008). Mineral soil addition is widespread during peatlands reclamation for trafficability and crop yields (Saurich et al., 2019), which also creates abundant iron resource (Banik et al., 2016). A recent study also reported that high percentages of agricultural land correlated with high concentrations of Fe in river water (Palviainen et al., 2015). According to our study, the N-induced microbial reduction of Fh-OC would increase dissolved iron and DOC release from precipitation of iron and organic carbon in long-term fertilized agricultural peatlands. Increased dissolved Fe could form dissolved complexes with phenolic (Wan et al., 2018),

hence creating the potential for greater exports of DOC and Fe from agricultural peatlands to aquatic systems.

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Table

Table1 Soil properties of agricultural and natural peatlands.

	TN ($g\ kg^{-1}$)	SOC (%)	TP ($g\ kg^{-1}$)	SRO ($g\ kg^{-1}$)	BD ($g\ cm^{-3}$)	NH_4^+-N ($mg\ kg^{-1}$)†	pH‡
Agricultural Peatlands	14.5±1.53	27.22±0.36	1.19±0.13	18.03±1.20	1.60±0.12	115-225	6.0
Natural Peatlands	18.4±0.61	40.41±0.15	0.80±0.03	7.20±1.65	0.12±0.03	25-60	5.4

TN: soil total nitrogen, SOC: Soil organic carbon, TP: Soil total phosphorus, SRO: soil short ranged iron, BD: soil bulk density. These were measured according to method of Carter (1993). Values are means ± deviations (n=3).

† Average soil ammonium nitrogen contents (NH_4^+-N) from May to September, which are from Shi (2019).

‡Average soil pore water pH, which are from Shi et al. (2017).

Figure Captions

Figure 1 Characteristics of microbial reduction of Fh-OC in cultures under different N availability. (a) With microbes from agricultural peatlands. (b) With microbes from natural peatlands. k represents the reduction rate of Fh-OC, evaluated as changes of Fe(Ⅱ) (μmol h^{-1}) over 240 h using linear fit. N0, 1, 10, 50, 100, 400 represent N concentration treatments (mg kg^{-1}), respectively. Value and error bars represent mean ± standard deviations (n=3) Different letters represent significant differences among N treatments ($p<0.05$).

Figure 2 Comparison of microbial Fe reduction rates in Fh-OC at field scale under different N availability in agricultural and natural peatlands. The error bars represent mean±standard deviations (n=3). N0, 1, 10, 50, 100, 400 represent N concentration treatments (mg kg^{-1}), respectively. Different letters represent significant differences among N treatments ($p<0.05$).

Figure 3 Nonmetric multidimensional scaling (NMDS) predicts iron-reducing bacteria composition in cultures from agricultural and natural peatlands with different N availability. Red and blue represent agricultural and natural peatlands, respectively. N0, 1, 10, 50, 100, 400 represent N concentration treatments (mg kg^{-1}), respectively. Each contains three replicates.

Figure 4 Heat map analysis of the highly represented bacterial functional groups in incubation cultures from natural and agricultural peatlands. The blue denotes low relative abundance and the red denotes high relative abundance. The colour key for the Z score indicates correspondence between blue-red colouring and standard deviations from the mean abundance of each functional group. A: agricultural peatlands; P: Natural peatlands; N0, 1, 10, 50, 100, 400 represent nitrogen concentrations (mg kg^{-1}), each contains three replicates.

Figure1

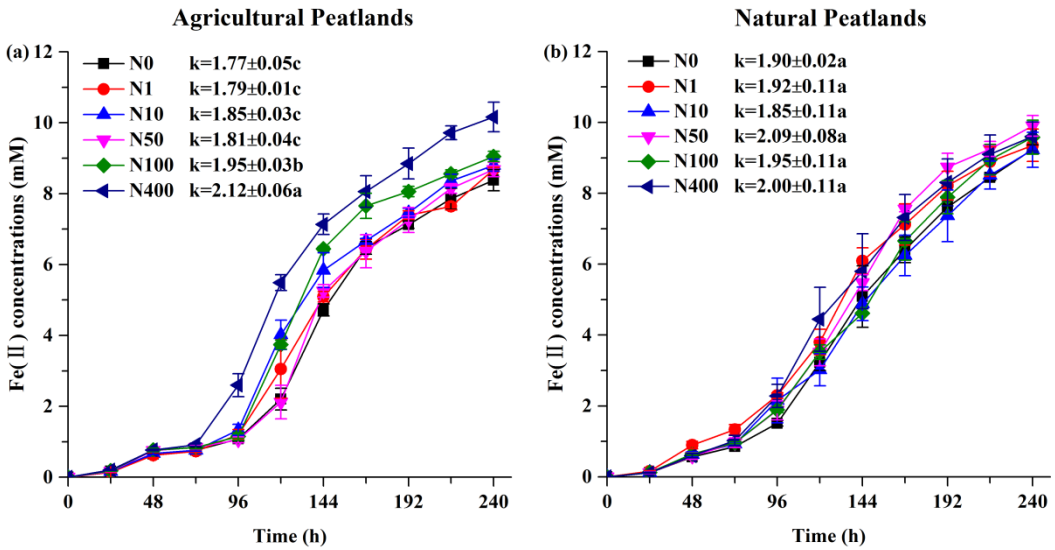
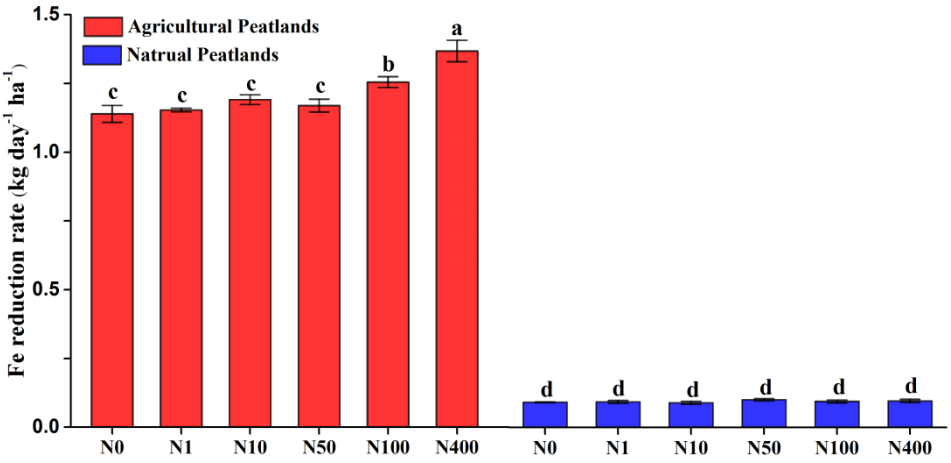
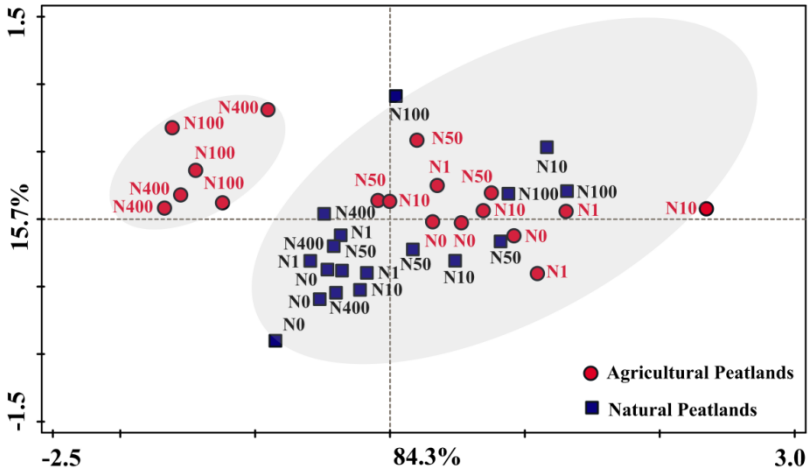

Agricultural Peatlands
(a)
N0 k=1.77±0.05c
N1 k=1.79±0.01c
N10 k=1.85±0.03c
N50 k=1.81±0.04c
N100 k=1.95±0.03b
N400 k=2.12±0.06a
Fe(Ⅱ) concentrations (mM)
Time (h)
Natural Peatlands
(b)
N0 k=1.90±0.02a
N1 k=1.92±0.11a
N10 k=1.85±0.11a
N50 k=2.09±0.08a
N100 k=1.95±0.11a
N400 k=2.00±0.11a
Fe(Ⅱ) concentrations (mM)
Time (h)

Figure 2


Agricultural Peatlands
Natrual Peatlands
Fe reduction rate (kg day-1 ha-1)
1.5
1.0
0.5
0.0
a
b
c
c
c
c
d
d
d
d
d
d
N0
N1
N10
N50
N100
N400
N0
N1
N10
N50
N100
N400

472 **Figure 3**



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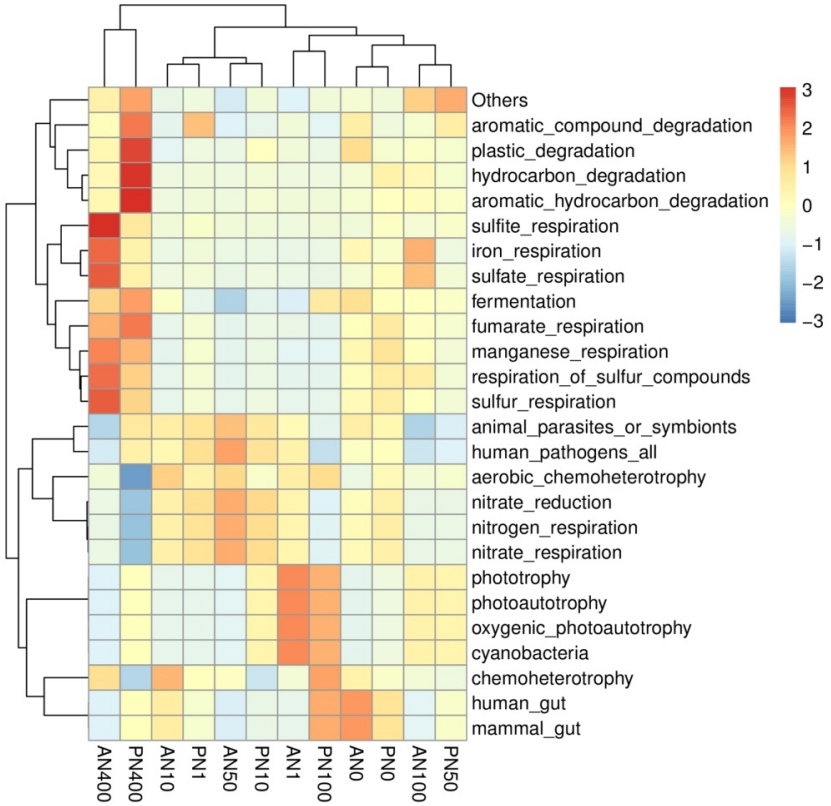
494 **Figure 4**



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